

CLAIMS

1. A detection method for detecting a variation in *GHI* effective to act as an indicator of GH dysfunction in an individual, which detection method comprises the steps of:
- 5 (a) obtaining a test sample comprising a nucleotide sequence of the human *GHI* gene from the individual; and
- (b) comparing the sequence obtained from the test sample with a standard sequence known to be that of the human *GHI* gene, wherein a difference between the
- 10 test sample sequence and the standard sequence indicates the presence of a variation (hereinafter "variant of *GHI*") effective to act as an indicator of GH dysfunction wherein the test sample is obtained from an individual exhibiting the following criterion:
- (i) growth failure, defined as a growth pattern [delineated by a series of height
- 15 measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9, p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch Dis Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the estimate being based upon the heights of the individual's parents.
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2. A method according to claim 1, wherein the test sample is obtained from an individual exhibiting at least one of the following further criteria:
- (ii) height velocity below the 25th centile for age; and/or
- (iii) bone age delay according to the Tanner-Whitehouse scale of at least two years
- 25 when compared with chronological age; and/or
- (iv) no other disorder known to cause inclusion in criteria (i) to (iii) above.
3. A method according to claim 2, wherein the bone age delay is in the range of from 2 to 4 years, when compared with chronological age.
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4. A method according to any preceding claim, wherein the individual exhibits normal results in a standard growth hormone function test.

5. A method according to any preceding claim, wherein the detection method comprises any sequencing method for determining the sequence of the *GHI* gene of an individual.

5 6. A method according to any preceding claim, wherein the detection method comprises PCR amplification of the *GHI* gene of the individual using (a) a *GHI* gene-specific fragment, being a fragment unique to the *GHI* gene whose sequence is not found in the four other paralogous (non-*GHI*) genes in the GH cluster, and (b) one or more *GHI* gene-specific primers which cannot bind to the homologous flanking
10 regions in the four other paralogous (non-*GHI*) genes in the GH cluster.

7. A method according to any preceding claim, wherein the detection method comprises PCR amplification of the entire *GHI* gene of the individual and nested PCR of overlapping constituent fragments of the *GHI* gene of the individual.
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8. A method according to any preceding claim, wherein the detection method comprises PCR amplification of all or a fragment of genomic DNA spanning the Locus Control Region of the *GHI* gene.

20 9. A method according to any preceding claim, wherein the detection method comprises mutational screening of all or a fragment of the individual's *GHI* gene by DHPLC.

10. A detection method for detecting a variation in *GHI* effective to act as an indicator of GH dysfunction in a individual, which detection method comprises the steps of:

- (a) obtaining a test sample comprising a nucleotide sequence of the human *GHI* gene from the individual; and
(b) comparing the sequence obtained from the test sample with a standard
30 sequence known to be that of the human *GHI* gene, wherein a difference between the test sample sequence and the standard sequence indicates the presence of a variation (hereinafter "variant of *GHI*") effective to act as an indicator of GH dysfunction which detection method further comprises

- (c) PCR amplification of the *GHI* gene of the individual using (a) a *GHI* gene-specific fragment, being a fragment unique to the *GHI* gene whose sequence is not found in the four other paralogous (non-*GHI*) genes in the GH cluster, and (b) one or more *GHI* gene-specific primers which cannot bind to the homologous flanking regions in the four other paralogous (non-*GHI*) genes in the GH cluster.

11. A detection method according to any preceding claim, which detection method further comprises the use of one or more primer(s) selected from:

- CTC CGC GTT CAG GTT GGC (GH1DF);
- 10 AGG TGA GCT GTC CAC AGG (GH1DR);
- GGG CAA CAG TGG GAG AGA AG (GH2DF);
- CCT CCA GGG ACC AGG AGC (GH2DR);
- CAT GTA AGC CCA GTA TTT GGC C (GH3DF);
- CTG AGC TCC TTA GTC TCC TCC TCT (GH3DR);
- 15 GAC TTT CCC CCG CTG GGA AA (GH4DF);
- GGA GAA GGC ATC CAC TCA CGG (GH4DR);
- TCA GAG TCT ATT CCG ACA CCC (GH5DF);
- GTG TTT CTC TAA CAC AGC TCT C (GH5DR);
- TCC CCA ATC CTG GAG CCC CAC TGA (GH6DF)
- 20 CGT AGT TCT TGA GTA GTG CGT CAT CG (GH6DR);
- TTC AAG CAG ACC TAC AGC AAG TTC G (GHD7F);
- CTT GGT TCC CGA ATA GAC CCC G (GH7DR);
- GTGCCCCAAGCCTTTCCC (LCR15: 1159-1177);
- TGTCAGATGTTTCAGTTCATGG (LCR13: 1391-1412);
- 25 CCTCAAGCTGACCTCAGG (LCR25: 1346-1363);
- GATCTTGGCCTAGGCCTCG (LCR23: 1584-1602);
- LCR 5A (5' CCAAGTACCTCAGATGCAAGG 3');
- LCR 3.0 (5' CCTTAGATCTTGGCCTAGGCC 3');
- LCR 5.0 (5' CCTGTACCTGAGGATGGG 3');
- 30 LCR 3.1 (5' TGTGTTGCCTGGACCCTG 3');
- LCR 3.2 (5' CAGGAGGCCTCACAAGCC 3');
- LCR 3.3 (5' ATGCATCAGGGCAATCGC 3');
- GH1G5 (5' GGTACCATGGCTACAGGTAAGCGCC 3');

GH1G3 (5' CTCGAGCTAGAAGCCACAGCTGCCC 3');

BGH3 (5' TAGAAGGCACAGTCGAGG 3');

GH1R5 (5' ATGGCTACAGGCTCCCGG 3'); and

GH1R3 (5' CTAGAAGCCACAGCTGCCC 3').

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12. A variant of *GHI*, which differs from *GHI* and is detected by or is detectable by a method according to any preceding claim but was not detected by methods used hitherto, such as those reliant on patient selection criteria based primarily on absolute height.

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13. A variant of *GHI*, which variant is selected from those characterised as unpublished in Table 7B herein "Growth Hormone deficiency; *GHI* gene mutations and polymorphisms".

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14. A variant of *GHI* according to any preceding claim comprising a missense mutation.

15. A variant of *GHI* according to any preceding claim comprising a silent mutation which affects the activity of the signal peptide.

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16. A variant of *GHI* comprising one or more of the following *GHI* promoter mutations:

Promoter <u>mutation</u>	Associated <u>haplotype</u>
A→G -248	1
T→C -495	1
A→G -177	1
T→C -30 (TATA)	1
A→G -24	1
C→T -347, A→G -44	1
A→G +62	1
G→A -48, A→G -498	2
T→C -508	2
ΔGGGGG -57 to -61	2
ΔG -57	2

17. A protein or amino acid sequence encoded by a variant of *GHI* according to any of claims 12 to 16.

5 18. A human GH variant, which variant is selected from the following amino acid substitutions with respect to wild type/GH:

Met→Val -26; Thr→Ala -20; Leu→Pro -12; Leu→Pro -11; Phe→Leu 1; Ile→Val 4;
Asp→Asn 11; Gln→Arg 22; Asp→Val 26; Glu→Gly 30; Lys→Arg 41; Ser→Leu
43; Glu→Gly 56; Arg→Gly 64; Ser→Phe 71; Glu→Lys 74; Ser→Pro 85; Trp→Arg
10 86; Gln→Leu 91; Asp→Gly 107; Ser→Cys 108; Ser→Arg 108; Val→Ile 110;
Tyr→His 143; Ala→Val 155; Leu→Pro 163; Lys→Arg 168; Lys→Glu 168;
Thr→Ala 175; and Phe→Ser 176.

19. A human GH variant, selected from one or more of (locus on hGH in
15 parentheses):

Ile4Val: (N-terminal, within site 2);

Gln22Arg: (helix 1);

Lys41Arg: (loop 1);

Glu56Gly: (in loop region between helices 1 and 2, part of binding site 1);

20 Arg64Gly: (loop 2);

Lys168Arg: (helix 4);

Lys168Glu; and

Thr175Ala: (helix 4)

as defined with respect to wild type hGH.

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20. A human GH variant, which variant comprises the following amino acid substitution with respect to wild type hGH: Glu→Gly 30 [Figure 7, SEQ ID NO:...]

21. A screening method for screening an individual suspected of GH dysfunction,
30 which screening method comprises the steps of:

(a) obtaining a test sample comprising a nucleotide sequence of the human *GHI* gene from the individual; and

(b) comparing a region of the sequence obtained from the test sample with the corresponding region of a predetermined sequence
wherein the predetermined sequence is selected from a variant of *GHI* according to any of claims 12 to 16.

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22. A screening method according to claim 21, wherein the test sample comprises genomic DNA.

23. A screening method for screening an individual suspected of GH dysfunction,
10 which screening method comprises the steps of:

(a) obtaining a test sample comprising a nucleotide sequence of the human *GHI* gene or an amino acid sequence encoded thereby from the individual; and

(b) analysing the test sample for the presence of a variant of *GHI* or a GH variant or for the presence of one or more surrogate markers that are indicative of or correlated
15 to the presence of a variant of *GHI* or a GH variant,

wherein the variant of *GHI* or the GH variant exhibits at least one variation when compared to the wild type hGH sequence and is obtainable from a second test sample derived from an individual exhibiting the following criterion:

(i) growth failure defined as a growth pattern [delineated by a series of height
20 measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9, p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch. Dis. Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the estimate being based upon the heights of the individual's parents.

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24. A screening method according to any one of claims 21 to 23, comprising:

(a) obtaining a first test sample from an individual; and

(b) comparing the *GHI* gene or *GHI* transcript, or fragment therefrom (*eg* cDNA), in the first test sample to the corresponding gene, transcript or fragment of a *GHI* variant
30 obtainable from a second test sample derived from an individual exhibiting the following criterion:

(i) growth failure defined as a growth pattern [delineated by a series of height measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9,

p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch. Dis. Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the estimate being based upon the heights of the individual's parents.

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25. A screening method according to claim 24, wherein the second test sample is obtainable from an individual exhibiting at least one of the following further criteria:

(ii) height velocity below the 25th centile for age; and/or

(iii) bone age delay according to the Tanner-Whitehouse scale of at least two years

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when compared with chronological age; and/or

(iv) no other disorder known to cause inclusion in criteria (i) to (iii) above.

26. A screening method according to any of claims 21 to 25 in which simultaneous screens are used either for multiple known mutations or for all possible mutations by
15 hybridization of a labelled sample of DNA (cDNA or genomic DNA derived from the individual) to micro-arrays of mutation-specific oligonucleotide probes immobilised on a solid support.

27. A screening method according to claim 26 in which a chip technology is used,
20 wherein the chip is a miniature parallel analytical device.

28. A kit suitable for use in carrying out a screening method according to any of claims 21 to 27, which kit comprises:

(a) an oligonucleotide having a nucleic acid sequence corresponding to a region of a
25 *GHI* variant, which region incorporates at least one variation from the corresponding wild-type hGH gene sequence; and/or

(b) an oligonucleotide having a nucleic acid sequence corresponding to the wild-type hGH gene sequence in the region specified in (a); and, optionally,

(c) one or more reagents suitable for carrying out PCR for amplifying desired regions
30 of the individual's DNA.

29. A kit according to claim 28, wherein the *GHI* variant comprises at least one of the variants claimed in claims 12 to 16.

30. A kit according to claim 28 or claim 29, wherein kit component (a) comprises a plurality of said oligonucleotides immobilised on a solid support.
- 5 31. A kit suitable for use in carrying out a detection method in which the variant is at least one of the variants claimed in claims 12 to 16.
32. A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:
- 10 (a) obtaining a test sample comprising an amino acid sequence encoded by the human *GHI* gene of the individual; and
- (b) analysing the test sample for the presence of a GH variant wherein the GH variant is selected from those according to any one of claims 17 to 20.
- 15 33. A screening method according to claim 32, wherein the analysis step (b) is selected from one or more of: conventional protein sequencing methods (such as mass spectroscopy, micro-array analysis, pyrosequencing, *etc*), and/or antibody-based methods of detection (*eg* ELISA).
- 20 34. An isolated, purified or recombinant nucleic acid sequence selected from:
- (a) a sequence comprising a variant of *GHI* according to any of claims 12 to 16 or encoding a GH variant according to any of claims 17 to 20
- (b) a sequence substantially homologous to or that hybridises to sequence (a) under stringent conditions; or
- 25 (c) a sequence substantially homologous to or that hybridizes under stringent conditions to the sequence (a) or (b) but for the degeneracy of the genetic code; or
- (d) an oligonucleotide specific for any of the sequences (a), (b) or (c).
- 30 35. A vector comprising a nucleic acid sequence according to claim 34.
36. A host cell comprising a vector according to claim 35, such as a bacterial host cell.

37. A process for preparing a variant of *GHI* according to any of claims 12 to 16, which process comprises:

- (i) culturing a host cell according to claim 36; and
- (ii) recovering from the culture medium the variant of *GHI* thereby produced.

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38. An amino acid sequence encoded or expressed by a sequence, vector, or cell as defined in any of claims 34 to 37 in culture medium.

39. A composition comprising a variant of *GHI* or a GH variant according to any of claims 12 to 16 or 17 to 20, respectively, in association with a pharmaceutically acceptable carrier therefor.

40. Use of a variant of *GHI* or a GH variant according to any of claims 12 to 16 or 17 to 20, respectively, for a therapeutic, diagnostic or detection method.

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41. Use according to claim 40 selected from one of more of: determining binding defects; determining pituitary storage defects; determining susceptibility to a disease, such as diabetes, obesity or infection; treating acromegaly or gigantism conditions associated with lactogenic, diabetogenic, lipolytic and protein anabolic effects; conditions associated with sodium and water retention; metabolic syndromes; mood and sleep disorders; and diagnosing GH dysfunction.

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42. Use according to claim 40 of one or more of the variants according to any of claims 12 to 16 in gene therapy.

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43. Use according to claim 40 of one or more of the variants according to any of claims 17 to 20 in protein therapy.

44. Use of a variant of GH1 or GH variant according to any of claims 12 to 16 or 17 to 20 respectively, in the preparation of a medicament, diagnostics composition or kit, or detection kit.

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